

Supplementary Information for

“Engineering modular and tunable genetic amplifiers for scaling transcriptional signals in cascaded gene networks”

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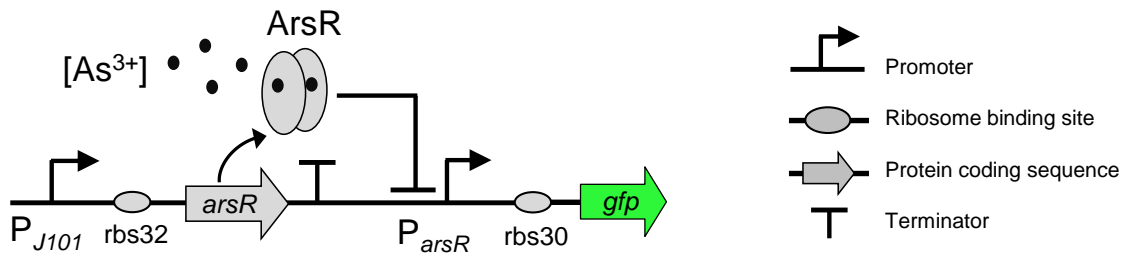
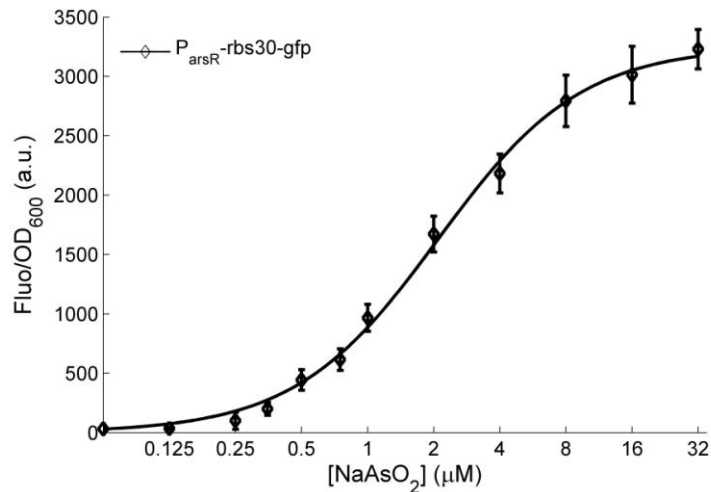
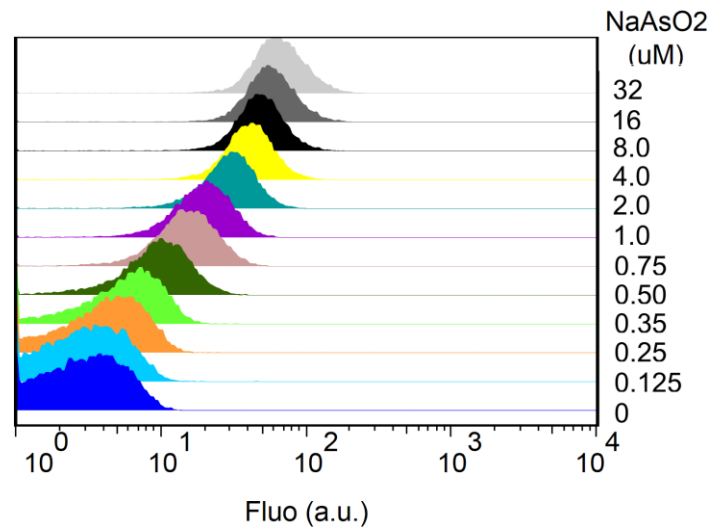
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Figure S1: Design and characterization of the arsenic responsive sensor. (A) The architecture of the arsenic responsive sensor. The arsenic sensor is designed with the arsenite responsive ArsR expressed under control of a constitutive promoter J105 and with *gfp* fused to the ArsR repressed promoter P_{arsR} as the output reporter. (B) The fitted transfer function of the arsenic input sensor against experimental data (Table S1). (C) The single cell flow cytometry data shows the cellular responses of the arsenic sensor induced by varied arsenic concentrations (0, 0.125, 0.25, 0.35, 0.5, 0.75, 1.0, 2.0, 4.0, 8.0, 16, 32 μM NaAsO₂).

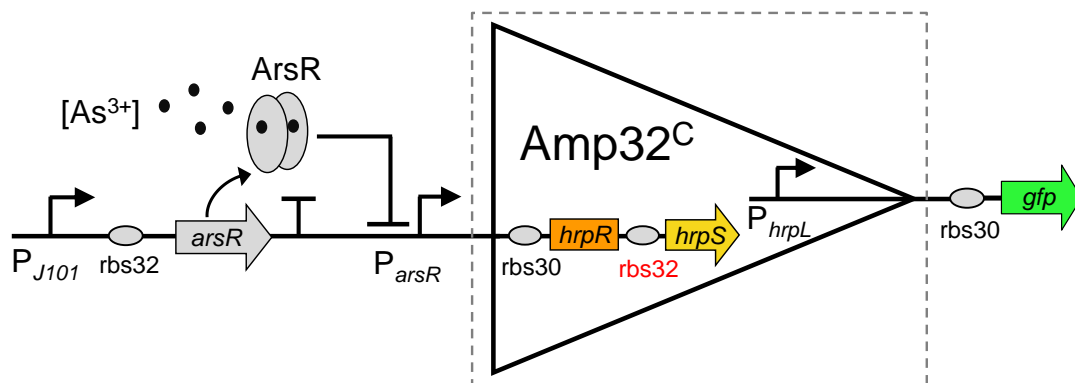
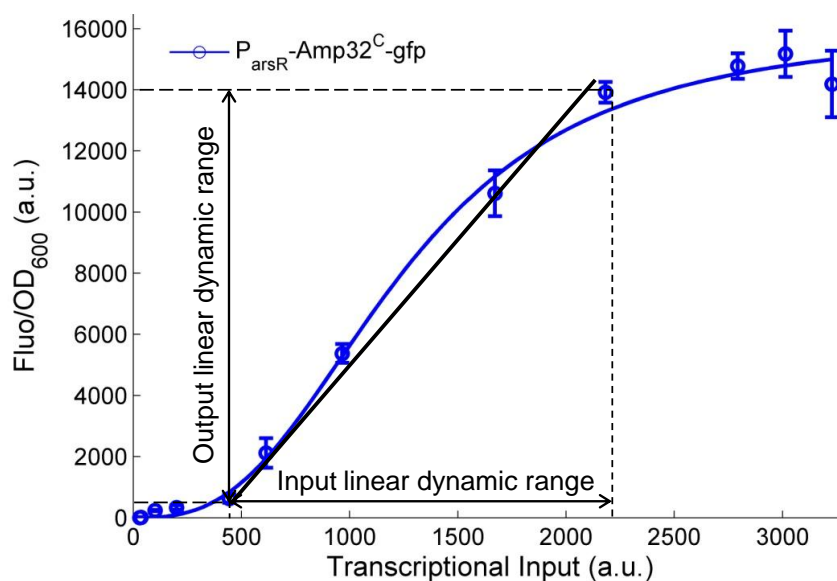
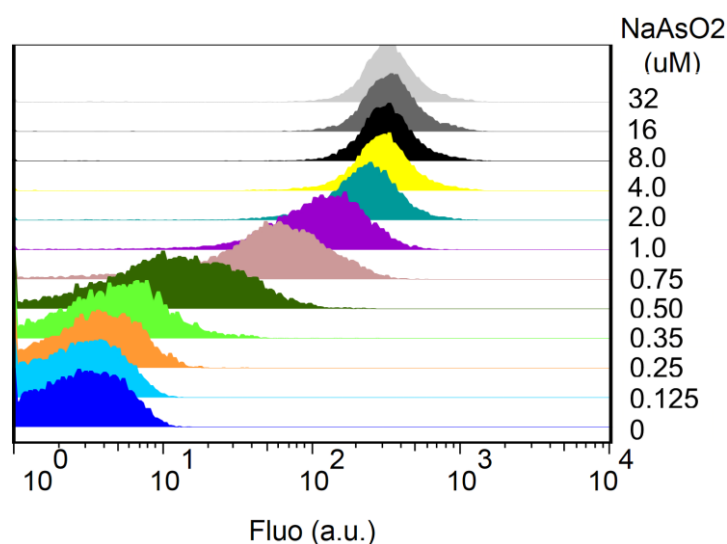
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Figure S2: Design and characterization of the arsenic sensor amplified by Amp32^C. (A) The arsenic sensor input is coupled to Amp32^C before cascaded to the *gfp* output. (B) The fitted transfer function of Amp32^C against the experimental data (Table S2). The device operating range used for linear fitting is also shown. (C) The single cell flow cytometry data shows the cellular responses of the amplified arsenic sensor induced by varied arsenic concentrations (0, 0.125, 0.25, 0.35, 0.5, 0.75, 1.0, 2.0, 4.0, 8.0, 16, 32 μ M NaAsO₂).

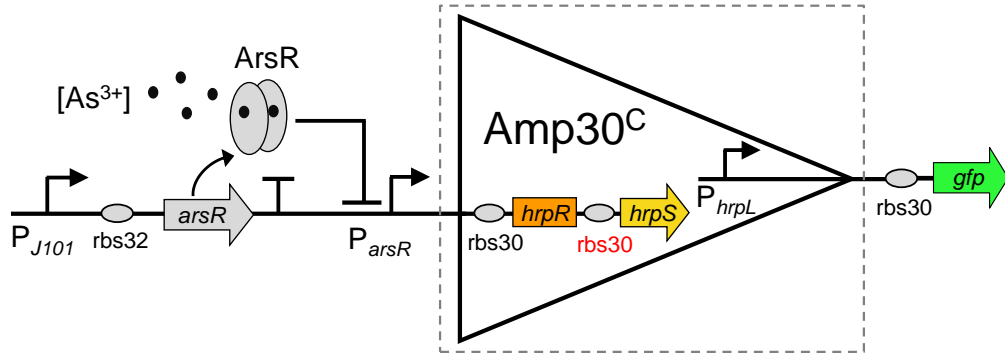
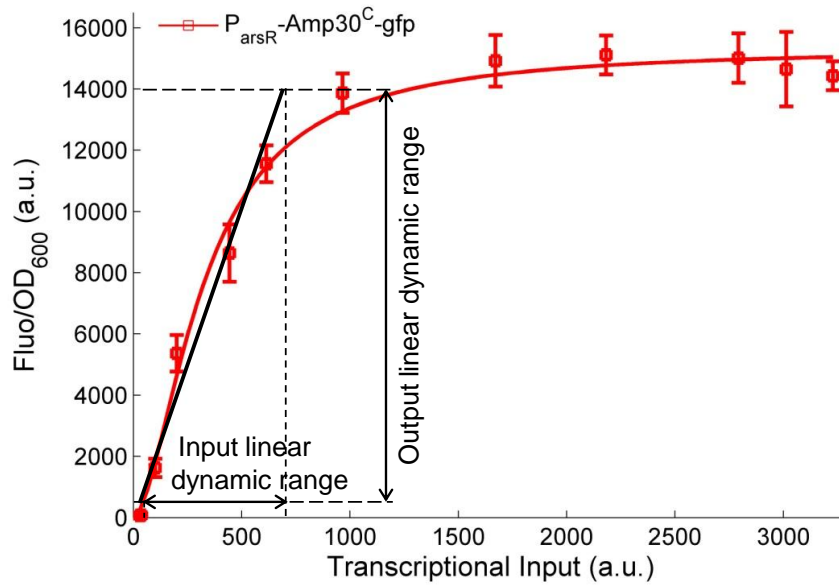
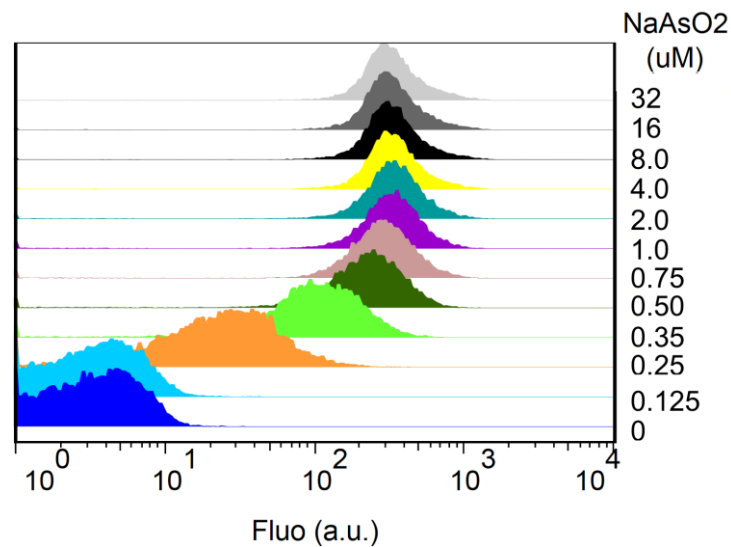
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Figure S3: Design and characterization of the arsenic sensor amplified by *Amp30^C*. (A) The arsenic sensor input is coupled to *Amp30^C* before cascaded to the *gfp* output. (B) The fitted transfer function of *Amp30^C* against the experimental data (Table S2). The device operating range used for linear fitting is also shown. (C) The single cell flow cytometry data shows the cellular responses of the amplified arsenic sensor induced by varied arsenic concentrations (0, 0.125, 0.25, 0.35, 0.5, 0.75, 1.0, 2.0, 4.0, 8.0, 16, 32 μM $NaAsO_2$).

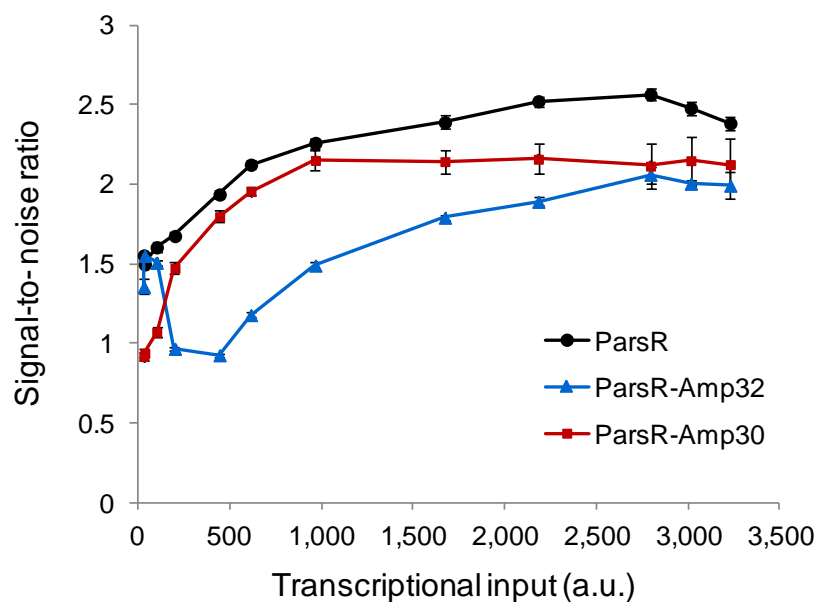


Figure S4: The signal-to-noise ratios of the arsenic input sensor without amplification and with amplification by Amp32^C and Amp30^C. The signal-to-noise ratio (SNR) is calculated as the ratio of the sample fluorescent mean to the standard deviation of the cell population from the single cell flow cytometry assay (Fig. S1C - S3C), i.e. $SNR = \mu/\sigma^1$. Data are means and s.d. for three replicates.

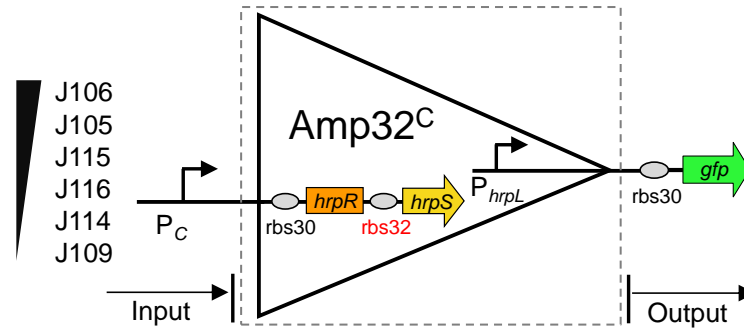
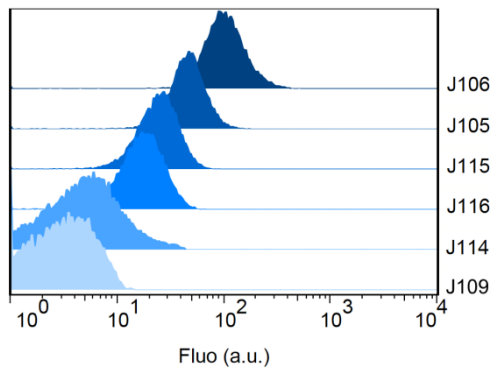
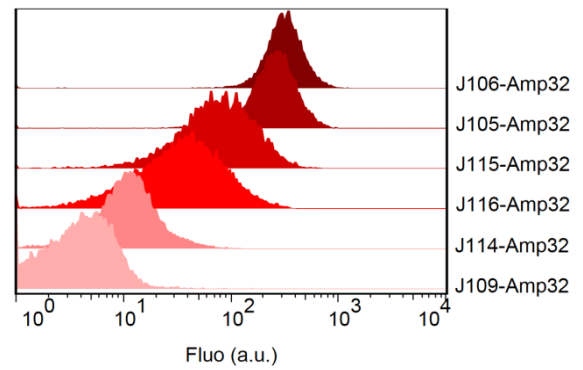
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Figure S5: Single cell characterization of Amp32^C with constitutive promoter inputs. (A) Schematic showing the fixed gain amplifier Amp32^C with constitutive promoter as the signal input. The single cell flow cytometry data shows the cellular responses of the 6 constitutive promoters (B) and those amplified by Amp32^C (C). The results demonstrate a consistent enhancing effect of the amplifier across the whole cell population with modest cell to cell variability being evident.

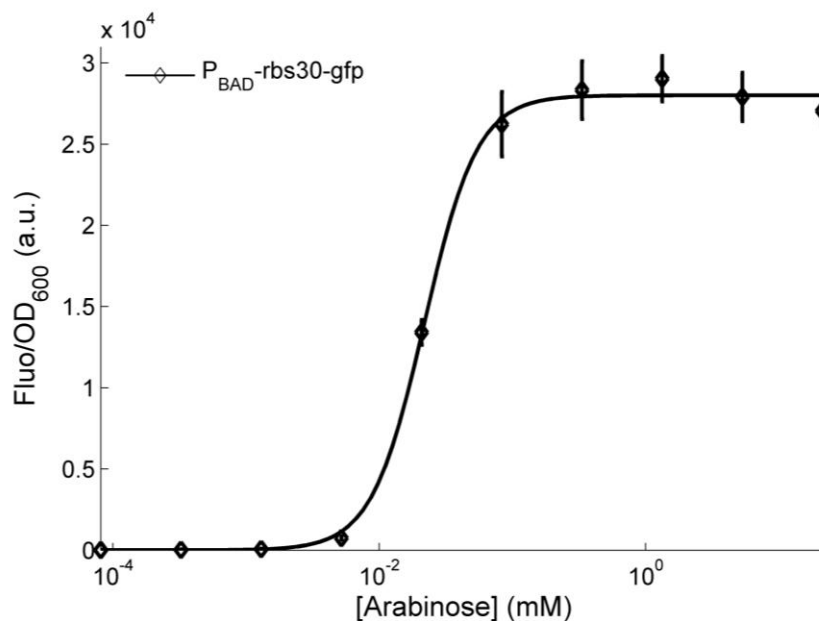


Figure S6: The transfer function of the characterised P_{BAD} promoter. The data were collected in *E. coli* TOP10 in LB media 5 hrs after induction by varied arabinose concentrations (0, 1.3×10^{-3} , 5.2×10^{-3} , 2.1×10^{-2} , 8.3×10^{-2} , 0.33, 1.33, 5.32 and 21.2 mM) and fitted to inducible promoter transfer function equation S2 (Table S1).

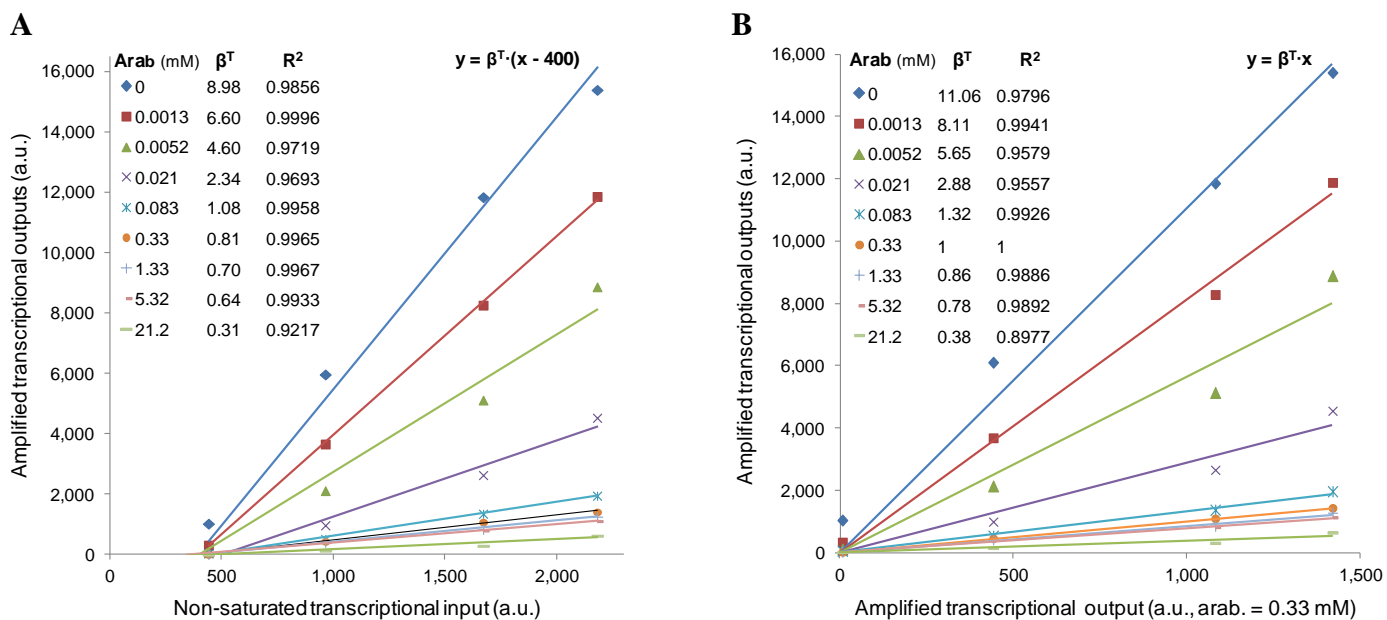


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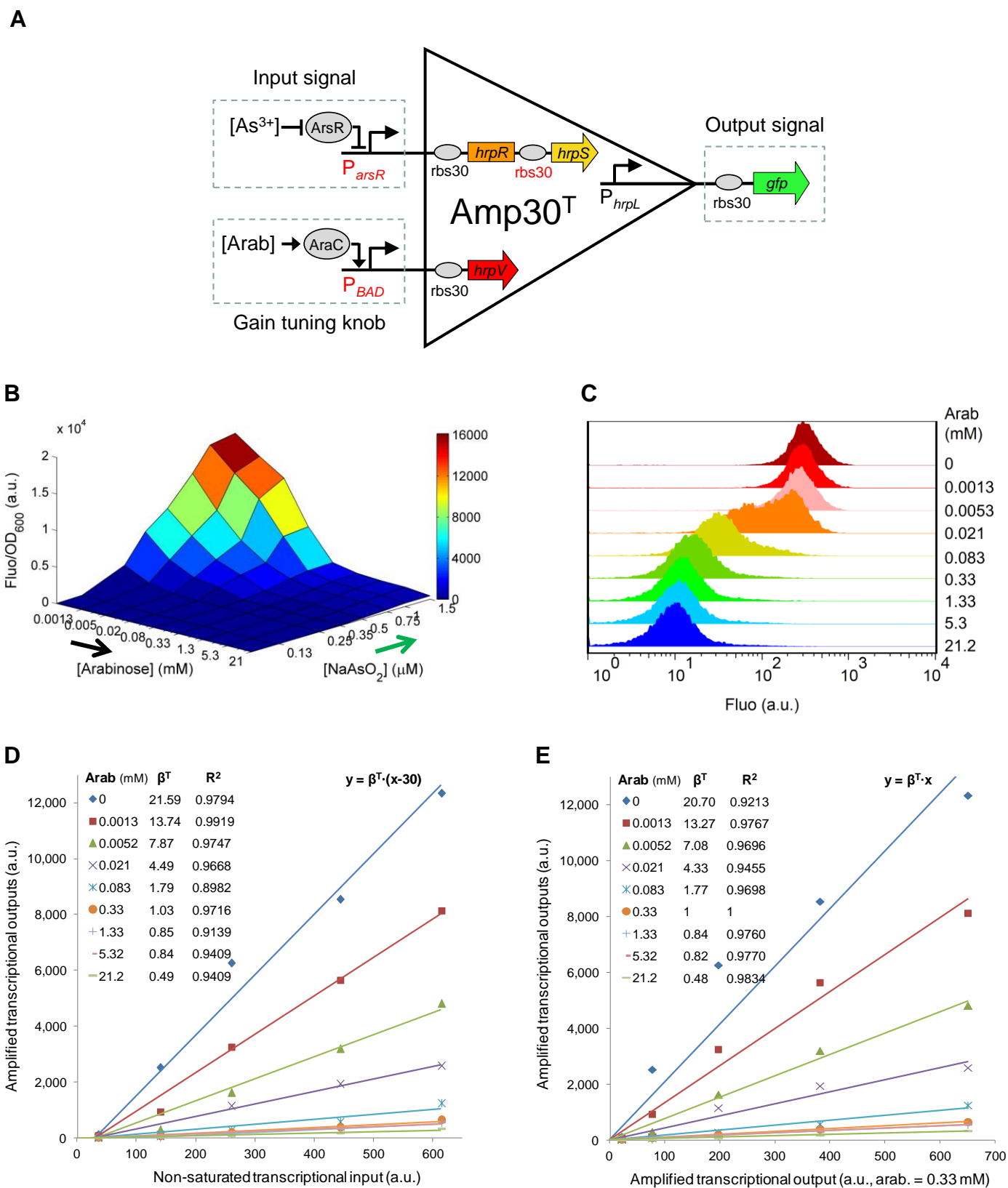


Figure S8: Engineering and characterization of the gain-tunable amplifier Amp30^T. (A)

The architecture of the tunable amplifier Amp30^T which contains three terminals corresponding to the Signal input, Gain tuning knob and Signal output respectively. The arsenic sensor is connected to the first terminal as the input signal. The arabinose inducible promoter P_{BAD} is

connected to the second terminal to control the amplification gain while *gfp* as the output reporter. **(B)** Steady state response of the amplifier as in **a** under 72 combinations of two input inductions (0, 0.125, 0.25, 0.35, 0.5, 0.75, 1.0, 1.5 μM NaAsO₂ by 0, 1.3×10^{-3} , 5.2×10^{-3} , 2.1×10^{-2} , 8.3×10^{-2} , 0.33, 1.33, 5.32 and 21.2 mM arabinose) measured by fluorometry. Data are means and s.d. for three replicates. **(C)** The single cell flow cytometry data shows the cellular responses of the amplified arsenic sensor induced under 1.5 μM NaAsO₂ and varied arabinose concentrations. **(D)** Scatter plot showing the linearity between the non-saturated inputs and their amplified outputs of Amp30^T under different gains as in **B** by fitting to a linear model. **(E)** Scatter plot showing the linearity between the amplified outputs of different gains as in **B**.

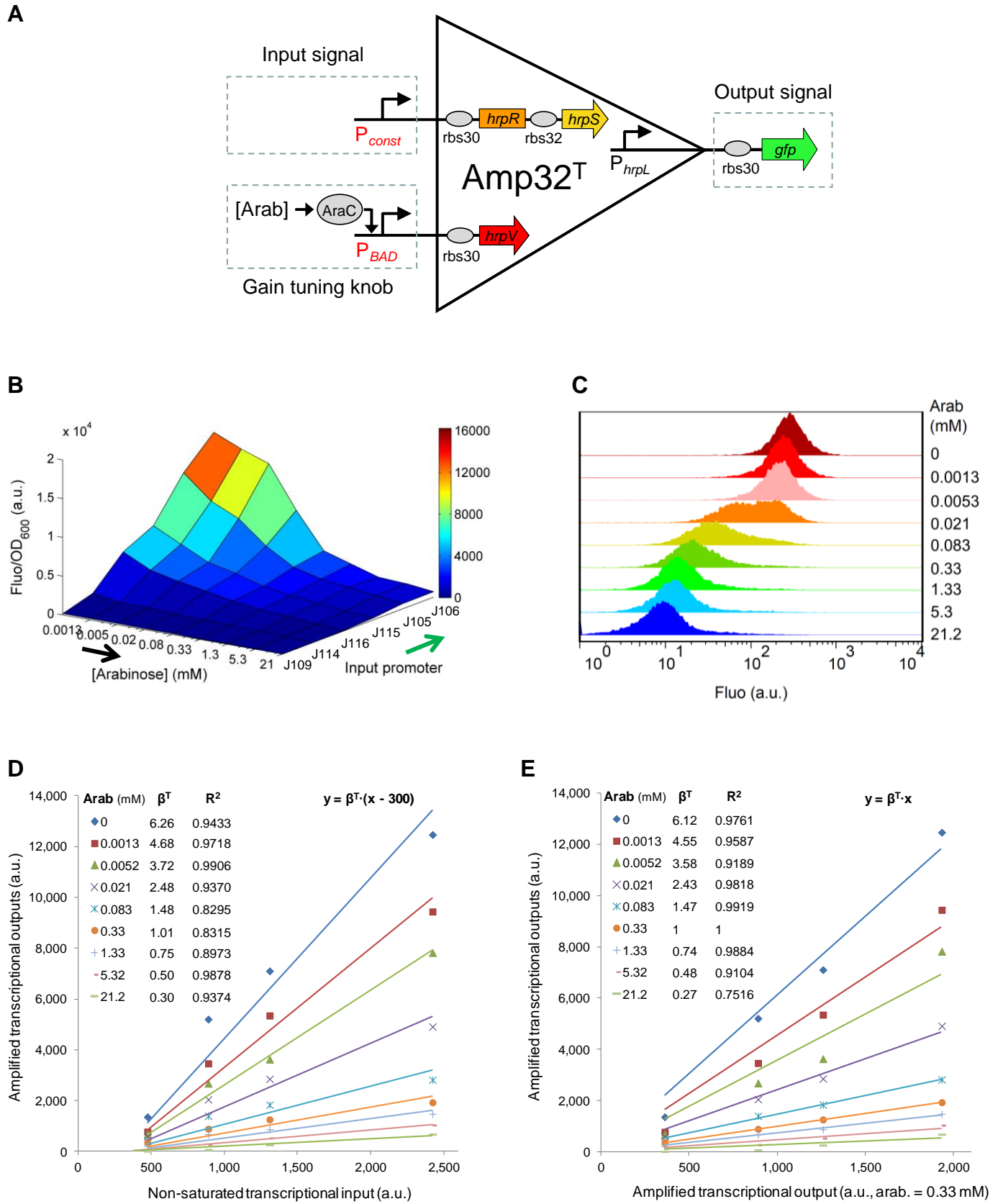


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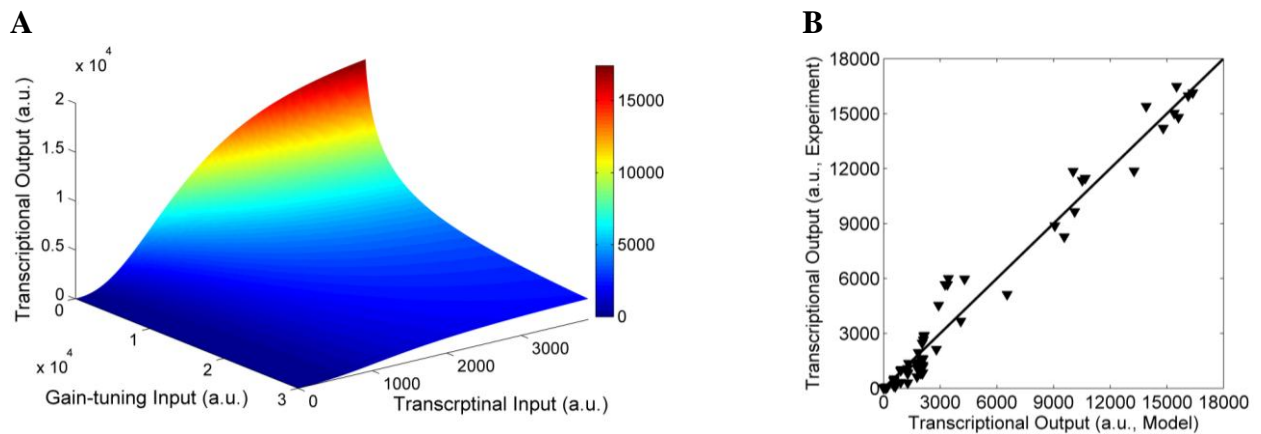


Figure S10: Amp32^T transfer function and comparison between model and experimental data. (A) The parameterised transfer function obtained by fitting to the experimental data (Fig. 4B, Table S2). (B) The Pearson correlation coefficients between the model and experimentally characterised responses of Amp32^T are 0.9863.

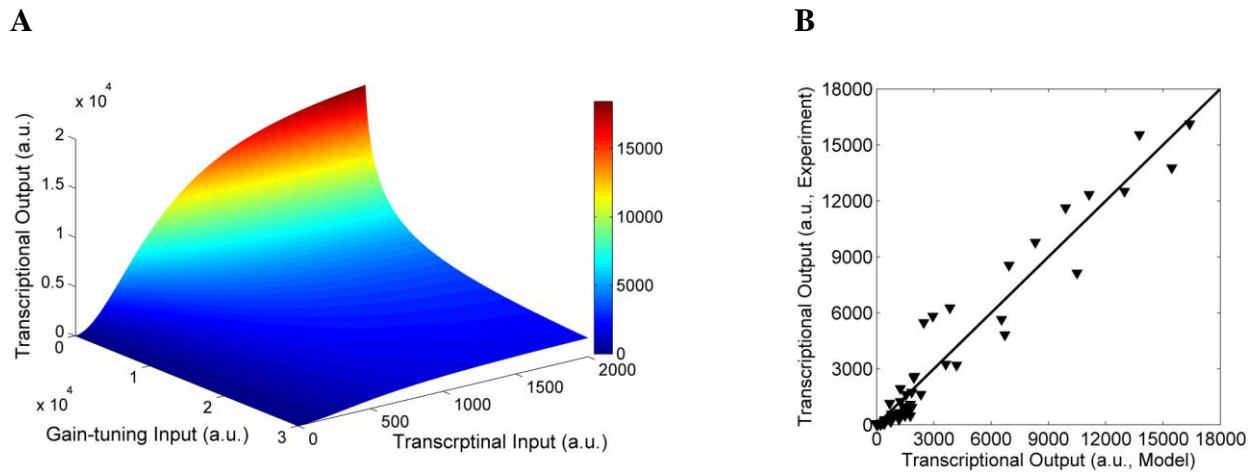


Figure S11: Amp30^T transfer function and comparison between model and experimental data. (A) The parameterised transfer function obtained by fitting to the experimental data (Fig. S8B, Table S2). (B) The Pearson correlation coefficients between the model and experimentally characterised responses of Amp30^T are 0.9736.

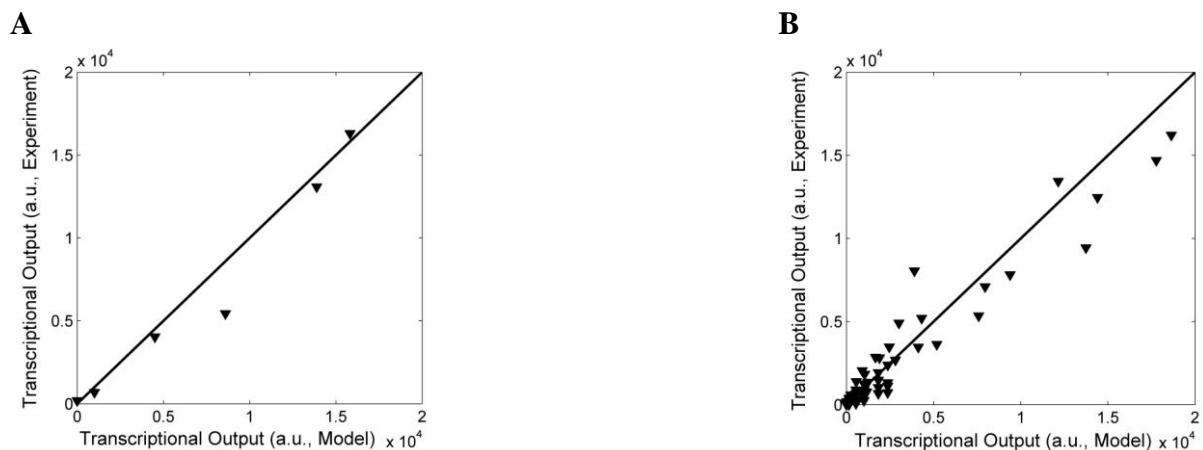


Figure S12: Comparison between amplifier model prediction and experimental data. (A) The Pearson correlation coefficient between the model predicted and experimentally characterised responses of the gain-fixed Amp32^C with the 6 constitutive promoters as inputs (Fig. 3D) is 0.9807. (B) The Pearson correlation coefficient between the model predicted and experimentally characterised responses of the gain-tunable Amp32^T with the 6 constitutive promoters as inputs (Fig. S9B) is 0.9643.

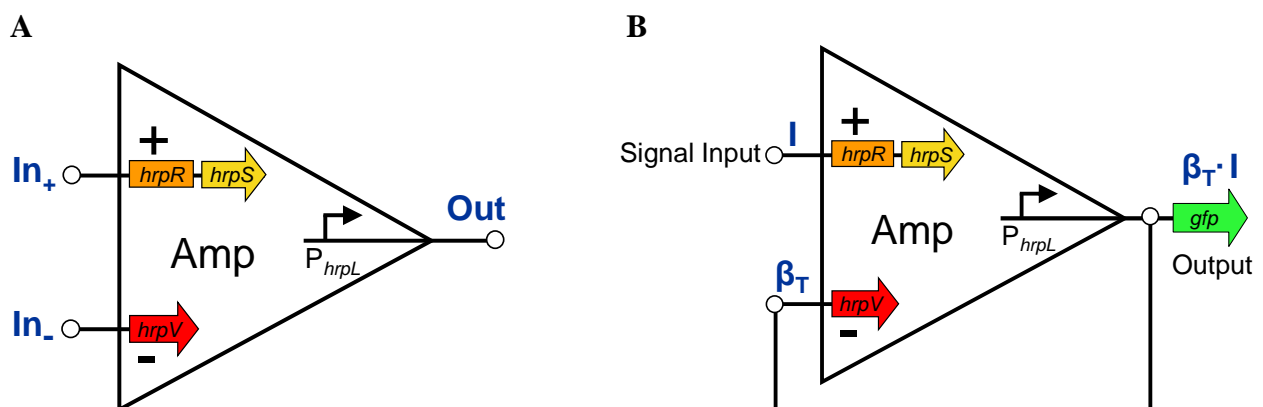
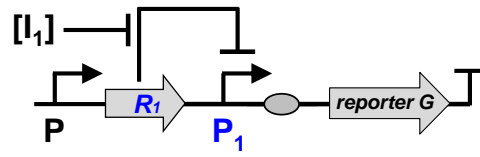


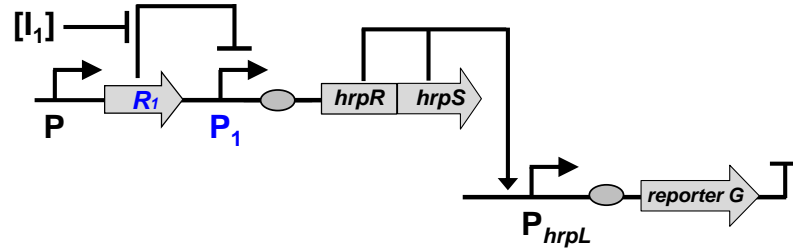
Figure S13: Schematics showing the potential applications of the tunable gain amplifier.

(A) The device can be applied as a differential amplifier² (operational amplifier) with the output transcriptional signal is in proportion to the difference between the signal input and gain-tuning control input ($\Delta[Output] = a \cdot \Delta[In_+] - b \cdot \Delta[In_-]$). (B) The device can be potentially applied to construct a proportional negative feedback circuit to adaptively tune the output signal to stay at a constant level, thus exempted from the impact of environmental context variations on the signal input³.

A



B



C

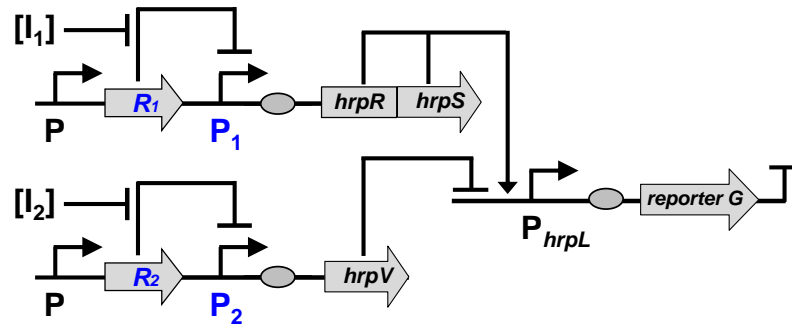


Figure S14: Schematics showing the architectures of the inducible negatively regulated promoter P_1 (A), the gain-fixed amplifier (B), and the gain-tunable amplifier (C).

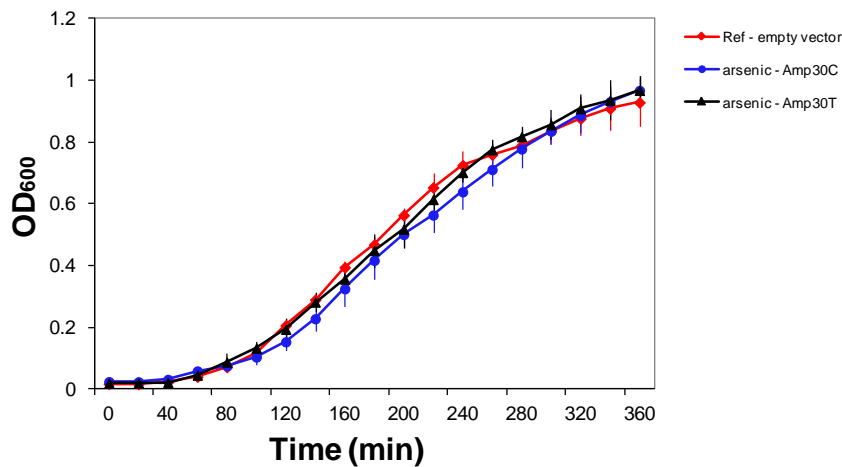


Figure S15: The growth curves of the genetic amplifiers ($Amp30^C$ and $Amp30^T$) engineered in this study compared to cells carrying the host plasmid alone show that the devices did not impose any observable growth burden on the *E. coli* host.

Host TOP10 cells containing various circuit constructs were used: one reference carrying the empty plasmid pSB3K3 alone (Ref – empty vectors), one carrying the plasmid with the functional $Amp30^C$ (pBW103ParsR- $Amp30^C$) using arsenic sensor as the input (Fig. 2, induced with 16 μ M NaAsO₂), and one carrying the plasmid with the functional $Amp30^T$ (pBW301ParsR- $Amp30^T$) using arsenic sensor and P_{BAD} as the inputs (Fig. S8, induced with 16 μ M NaAsO₂ and 0.021 mM arabinose). The cells were grown in a 96 well microplate in fluorometer with shaking (200 rpm) for 6 hours. The absorbance (OD₆₀₀) was read every 20 min. The data were the average of three repeats from the three colonies of each strain. Cells were grown in LB media at 37 °C. Error bars, s.d. (n = 3).

Supplementary Methods

Mathematical modelling and data fitting

Computational biochemical models were developed for individual genetic parts, and modules to abstract their behaviours for their future predictable assembly into larger synthetic biological systems. In this study, we focus on the average behaviour of the *E. coli* population to demonstrate the performance of the engineered circuits at steady state. First we used a simple linear mathematical formula ($y = k \cdot (x - b)$) to model the input-output relationship within the linear amplification dynamic range of the amplifier system. In addition, the ODEs-based deterministic model was used for accurately modelling the gene regulation and expression across the whole input or output range of the biochemical system. The following describes the derivation of the transfer function (TF) for each genetic circuit module and the experimental data fitting to these models.

1. Deriving transfer function of the inducible promoters

Figure S14A shows the exemplar architecture of the inducible promoter used in this study (*arsR-P_{arsR}* and *araC-P_{BAD}* promoters). The promoter P_1 is negatively regulated by its constitutively expressed repressor R_1 and is responsive to exogenous inducer I_1 to activate transcription of downstream reporter gene G . The reporter gene expression can be modelled by⁴⁻⁶:

$$\frac{d[G]}{dt} = \alpha \cdot k_1 + \frac{k_1 \cdot [I_1]^{n_1}}{[I_1]^{n_1} + K_1^{n_1}} - d \cdot [G] \quad (S1)$$

where $\alpha \cdot k_1$ is the basal constitutive activity of the promoter, $k_1 \cdot [I_1]^n / ([I_1]^n + K_1^n)$ is the activity due to cooperative transcription activation by assuming the concentration of the repressor is constant to model the effect of varying the concentration of the inducer I_1 , and $d \cdot [G]$ is the constitutive degradation activity of protein G . K_1 and n_1 are the Hill constant and coefficient relating to the promoter-regulator/inducer interaction, k_1 is the maximum expression rate due to induction and α is a constant relating to the promoter basal level due to leakage ($0 \leq \alpha < 1$), and d is the degradation rate of G .

The steady state solution of equation S1 is given by

$$f([I_1]) = [G]_{ss} = k \cdot (\alpha + [I_1]^{n_1} / (K_1^{n_1} + [I_1]^{n_1})) \quad (S2)$$

in which $k = k_i/d$ represents the maximum expression level due to induction. Equation S2 gives the reporter protein level at steady state for the inducible promoter P_1 and is also the TF of P_1 . We used this TF to fit the characterisation data of the arsenite (Fig. S1) and arabinose (Fig. S4) inducible promoters using the nonlinear least square fitting function in Matlab. The best fit coefficients (with 95% confidence bounds otherwise fixed at bound) are listed in Supplementary Table S1 and the parameterised TFs are plotted in Fig. S1B and Fig. S2B respectively against their experimental data.

2. Deriving transfer function of the gain-fixed amplifier (Amp^C)

Figure S14B shows the architecture of the gain-fixed amplifier in this study. *hrpL* promoter is synergistically activated by the heteromeric protein complex HrpRS, which are co-transcribed as a single operon. Based on the known mechanism underlying this hetero-regulated module⁶⁻⁷, the two bacterial enhancer-binding proteins form an active hetero-hexamer complex first to bind the UAS (upstream activation sequence) of *hrpL* to remodel the conformation of σ^{54} -RNAP-*hrpL* close promoter complex to an open one for the transcriptional activation. The amplifier TF can be described by the following Hill function curve with quasi-stationary approximation:

$$f([RS]_{ss}) = k_c \cdot \frac{([RS]_{ss}/K_{RS})^{n_{RS}}}{1 + ([RS]_{ss}/K_{RS})^{n_{RS}}} \quad (S3)$$

in which K_{RS} and n_{RS} are the Hill constant and coefficient for HrpRS co-activator. $[RS]_{ss}$ is the steady level of HrpRS, whose level is under the control of the inducible promoter P_1 as indicated by equation S2 or a constitutive promoter. k_c is the maximum output level of the amplifier at steady state.

The TF was parameterised by fitting to the experimental data of the two amplifiers Amp32^C and Amp30^C (Fig. 2B). The best fit coefficients by nonlinear least square optimisation were obtained as shown in Supplementary Table S2. The parametrised TFs are plotted in Fig. S2B and Fig. S3B respectively against the experimental data. Figure S12A shows the linear correlation between predicted and experimentally characterised responses of the amplifier with alternate constitutive promoter inputs (Fig. 3D).

3. Deriving transfer function of the gain-tunable amplifier (Amp^T)

Figure S14C shows the architecture of the gain-tunable genetic amplifier in this study. The device is designed on the basis of the three regulatory components HrpR, HrpS, HrpV and one output regulated promoter *hrpL*. The *hrpL* promoter is activated by the heteromeric protein complex HrpRS, transcribed from a single operon, while negatively regulated by the HrpV protein through a direct interaction with HrpS⁷⁻⁸. As shown, the amplifier can be characterised under two inducible promoters P₁ (in response to inducer I₁) as the input signal and P₂ (in response to inducer I₂) as the gain tuning input. Following quasi-stationary approximation, the device TF can be modelled by

$$f([RS]_{ss}, [V]_{ss}) = k_T \cdot \frac{([RS]_{ss}/K_{RS})^{n_{RS}}}{1 + ([RS]_{ss}/K_{RS})^{n_{RS}}} \cdot \frac{1}{1 + ([V]_{ss}/K_V)^{n_V}} \quad (S4)$$

in which K_{RS} and n_{RS} are the Hill constant and coefficient for HrpRS co-activator. $[RS]_{ss}$ is the steady level of HrpRS, whose level is under the control of the inducible promoters P₁ as indicated by equation S2 or a constitutive promoter. K_V and n_V are the Hill constant and coefficient for HrpV repressor. $[V]_{ss}$ is the steady level of HrpV, whose level is under the control of the inducible promoter P₂ as indicated by equation S2. k_T is the maximum output level of the amplifier at steady state.

The characterisation data of the Amp32^T and Amp30^T using arsenic sensor as the signal input and P_{BAD} promoter as the gain-tuning input (Fig. 4B, Figure S8B) were fitted to this transfer function model and the results are listed in Supplementary Table S2. The parametrised TFs are plotted in Fig. S10A and Fig. S11A respectively. There are high linear correlation between model prediction and the experimental data as shown in Fig. S10B and Fig. S11B. Figure S12B show the linear correlation between the model predicted and experimentally characterised responses of the amplifier Amp32^T with alternate constitutive promoter inputs (Fig. S9B).

4. Deriving device linear dynamic range

The device linear dynamic range in Figure S2B and S3B is derived according to the difference (non-linearity) between the fitted Hill function and the fitted linear function for the experimental characterisation data. If non-linearity $|(Hill(x) - Linear(x))/Linear(x)| \leq 15\%$, the corresponding transcriptional input (x) and output ($Linear(x)$) is considered within the device's input and output linear dynamic ranges.

Table S1: The best fits of the characterised promoter responses. Data shown are with 95% confidence bounds.

Promoter Input	k (a.u.)	n_I	K_I (μ M)	α	R^2
P _{J101} -rbs32- <i>arsR</i> -B0015-P _{arsR}	3235 \pm 146	1.332 \pm 0.171	2.088 \pm 0.299	5.069e-10	0.9972
<i>araC</i> -P _{BAD}	2.801e4 \pm 0.069e4	2.195 \pm 0.62	21.82 \pm 2.07	3.823e-9	0.9985

Table S2: The best fits for the characterised responses of the amplifiers. Data shown are with 95% confidence bounds.

Amplifier	k_C or k_T (a.u.)	n_{RS}	K_{RS} (a.u.)	n_V	K_V (a.u.)	R^2
Amp32 ^C	1.602e4 \pm 0.135e4	2.811 \pm 0.62	1243 \pm 141	N/A	N/A	0.9964
Amp30 ^C	1.534e4 \pm 0.083e4	1.715 \pm 0.393	326.1 \pm 49.8	N/A	N/A	0.9932
Amp32 ^T	1.95e4 \pm 0.317e4	2.295 \pm 0.618	1540 \pm 296	0.8003 \pm 0.0917	2528 \pm 620	0.9725
Amp30 ^T	2.039e4 \pm 0.513e4	1.876 \pm 0.572	600.5 \pm 199.3	0.7936 \pm 0.1443	1961 \pm 777	0.9468

Table S3: Amp32^T (P_{arsR} as signal input and P_{BAD} as gain tuning input, Fig. 4B, D, E) linear correlation coefficient matrix between the unsaturated input and the outputs under different gains (indicated by varying arabinose concentrations)

		Amp32 ^T Outputs (Arabinose - mM)									
		Input	0	1.3×10 ⁻³	5.2 ×10 ⁻³	2.1 ×10 ⁻²	8.3 ×10 ⁻²	0.33	1.33	5.32	21.2
Outputs (Arabinose - mM)	Input	1	0.9983	0.9998	0.9893	0.9885	0.9987	0.9985	0.9985	0.9969	0.9658
	0	0.9983	1	0.997	0.9796	0.978	0.9944	0.9991	0.9957	0.9985	0.951
	1.3×10 ⁻³	0.9998	0.997	1	0.992	0.9913	0.9991	0.9972	0.999	0.9959	0.9708
	5.2 ×10 ⁻³	0.9893	0.9796	0.992	1	0.9996	0.9925	0.9802	0.993	0.9812	0.9929
	2.1 ×10 ⁻²	0.9885	0.978	0.9913	0.9996	1	0.993	0.9799	0.991	0.9783	0.9916
	8.3 ×10 ⁻²	0.9987	0.9944	0.9991	0.9925	0.993	1	0.9964	0.9966	0.9916	0.9709
	0.33	0.9985	0.9991	0.9972	0.9802	0.9799	0.9964	1	0.9943	0.9956	0.9504
	1.33	0.9985	0.9957	0.999	0.993	0.991	0.9966	0.9943	1	0.9972	0.975
	5.32	0.9969	0.9985	0.9959	0.9812	0.9783	0.9916	0.9956	0.9972	1	0.9569
	21.2	0.9658	0.951	0.9708	0.9929	0.9916	0.9709	0.9504	0.975	0.9569	1

Table S4: *p*-values of Amp32^T (P_{arsR} as signal input and P_{BAD} as gain tuning input, Fig. 4B, D, E) linear correlation coefficient matrix between the unsaturated input and the outputs under different gains (indicated by varying arabinose concentrations)

		Amp32 ^T Outputs (Arabinose - mM)									
		Input	21.2	5.32	1.33	0.33	8.3 ×10 ⁻²	2.1 ×10 ⁻²	5.2 ×10 ⁻³	1.3×10 ⁻³	0
Outputs (Arabinose - mM)	Input	0	0.0017	0.0002	0.0107	0.0115	0.0013	0.0015	0.0015	0.0031	0.0342
	21.2	0.0017	0	0.0030	0.0204	0.0220	0.0056	0.0009	0.0043	0.0015	0.0491
	5.32	0.0002	0.0030	0	0.0080	0.0087	0.0009	0.0028	0.0010	0.0041	0.0292
	1.33	0.0107	0.0204	0.0080	0	0.0004	0.0075	0.0198	0.0070	0.0188	0.0071
	0.33	0.0115	0.0220	0.0087	0.0004	0	0.0070	0.0201	0.0090	0.0217	0.0084
	8.3 ×10 ⁻²	0.0013	0.0056	0.0009	0.0075	0.0070	0	0.0036	0.0034	0.0084	0.0291
	2.1 ×10 ⁻²	0.0015	0.0009	0.0028	0.0198	0.0201	0.0036	0	0.0057	0.0044	0.0496
	5.2 ×10 ⁻³	0.0015	0.0043	0.0010	0.0070	0.0090	0.0034	0.0057	0	0.0028	0.0250
	1.3×10 ⁻³	0.0031	0.0015	0.0041	0.0188	0.0217	0.0084	0.0044	0.0028	0	0.0431
	0	0.0342	0.0491	0.0292	0.0071	0.0084	0.0291	0.0496	0.0250	0.0431	0

Table S5: Amp30^T (P_{arsR} as signal input and P_{BAD} as gain tuning input, Fig. S5B, D, E) linear correlation coefficient matrix between the unsaturated input and the outputs under different gains (indicated by varying arabinose concentrations)

		Amp30 ^T Outputs (Arabinose - mM)									
		Input	0	1.3×10 ⁻³	5.2 ×10 ⁻³	2.1 ×10 ⁻²	8.3 ×10 ⁻²	0.33	1.33	5.32	21.2
Outputs (Arabinose - mM)	Input	1	0.9915	0.997	0.9928	0.9859	0.9628	0.988	0.9843	0.9708	0.9726
	0	0.9915	1	0.9916	0.9815	0.9852	0.9455	0.973	0.9633	0.946	0.9516
	1.3×10 ⁻³	0.997	0.9916	1	0.9977	0.9948	0.9657	0.989	0.9892	0.9705	0.9742
	5.2 ×10 ⁻³	0.9928	0.9815	0.9977	1	0.9917	0.9759	0.9937	0.9966	0.9807	0.9838
	2.1 ×10 ⁻²	0.9859	0.9852	0.9948	0.9917	1	0.9433	0.9725	0.9806	0.9476	0.9535
	8.3 ×10 ⁻²	0.9628	0.9455	0.9657	0.9759	0.9433	1	0.9927	0.9813	0.9967	0.9982
	0.33	0.988	0.973	0.989	0.9937	0.9725	0.9927	1	0.994	0.9951	0.9966
	1.33	0.9843	0.9633	0.9892	0.9966	0.9806	0.9813	0.994	1	0.9884	0.9897
	5.32	0.9708	0.946	0.9705	0.9807	0.9476	0.9967	0.9951	0.9884	1	0.9994
	21.2	0.9726	0.9516	0.9742	0.9838	0.9535	0.9982	0.9966	0.9897	0.9994	1

Table S6: Amp32^T (P_C as signal input and P_{BAD} as gain tuning input, Fig. S6 B, D, E) linear correlation coefficient matrix between the unsaturated input and the outputs under different gains (indicated by varying arabinose concentrations)

		Amp32 ^T Outputs (Arabinose - mM)									
		Input	0	1.3×10 ⁻³	5.2 ×10 ⁻³	2.1 ×10 ⁻²	8.3 ×10 ⁻²	0.33	1.33	5.32	21.2
Outputs (Arabinose - mM)	Input	1.0000	0.9882	0.9914	0.9966	0.9891	0.9695	0.9838	0.9847	0.9969	0.9920
	0	0.9882	1.0000	0.9989	0.9924	0.9999	0.9952	0.9977	0.9997	0.9755	0.9614
	1.3×10 ⁻³	0.9914	0.9989	1.0000	0.9916	0.9995	0.9929	0.9987	0.9976	0.9823	0.9692
	5.2 ×10 ⁻³	0.9966	0.9924	0.9916	1.0000	0.9920	0.9757	0.9841	0.9907	0.9871	0.9806
	2.1 ×10 ⁻²	0.9891	0.9999	0.9995	0.9920	1.0000	0.9950	0.9984	0.9993	0.9775	0.9635
	8.3 ×10 ⁻²	0.9695	0.9952	0.9929	0.9757	0.9950	1.0000	0.9966	0.9964	0.9530	0.9329
	0.33	0.9838	0.9977	0.9987	0.9841	0.9984	0.9966	1.0000	0.9969	0.9734	0.9573
	1.33	0.9847	0.9997	0.9976	0.9907	0.9993	0.9964	0.9969	1.0000	0.9702	0.9551
	5.32	0.9969	0.9755	0.9823	0.9871	0.9775	0.9530	0.9734	0.9702	1.0000	0.9980
	21.2	0.9920	0.9614	0.9692	0.9806	0.9635	0.9329	0.9573	0.9551	0.9980	1.0000

Table S7. List of genetic parts used in this study.

Part name	Type and source	DNA sequence (5' – 3')
<i>hrpR</i>	Gene ^{6,9}	ATGAGTACAGGCATCGATAAGGACGTCCGAGAGTGTGGGGCGTAACTGCAT TATCAGCGGGTCATCAAATTGCAATGAATAGCGCGTTTCTGGATATGGACTT GCTGTTGTGCGGGGAAACCGGCACCGGCAAGGACACACTGGCCAACCGCATT CACGAGTTGTCCAGCAGGTCCGGACCCCTTTGTGGGCATGAACTGCGCCGCCA TTCCCGAGTCGCTGGCAGAGAGCCAGTTATTCGGTGTGGTCAACGGTGCATT CACCGGCGTATGCCGGGCTCGCGAGGGCTACATAGAGGCCCTCCAGTGGTGGC ACCTTGTACCTGGATGAAATCGACAGCATGCCGTTGAGCCTGCAAGCCAAAC TGCTGCGTGTGTGGAGAGTCGAGGTATCGAGCGTCTGGGCTCGACCGAATT TATCCCGGTGGATCTGCGGATCATTGCCTCGGCCAGCGGCCACTGGATGAA CTGGTGGAAACAAGGACTTTTCCGTCGCGACCTGTTTTTTCGGCTCAACGTGC TGACGCTTCACTTGCCAGCCTTGCGCAAACGTCGTGAACAGATCCTGCCATT GTTTCGACCAGTTCACCCAGGGTATCGCTGCCGAGTTCGGACGTCCCGCTCCT GCGCTGGACAGCGGGCGTGTGCAGCTGCTGCTCAGCCACGACTGGCCGGGCA ACATCCGCGAATTGAAGTCTGCGGGCAAGCGCTTCGTACTCGGCTTCCCTT GCTGGGCGCCGACCCGTGGAAGCGCTTGACCTGCCACGGGGCTGCGCACG CAAATGCGCATCATCGAGAAAATGCTCATCCAGGATGCCTTGAAGCGGCACA GGCACAATTTTCGACGCGGTGCTTCAGGAGTTGGAGTTGCCAAGACGCACCCT GTATCACCGCATGAAGGAAC TGGGAGTTGCAGCGCCGATCGCTGCGACGGCC GGGTCTAATAA
<i>hrpS</i>	Gene ^{6,9}	ATGAGTCTTGATGAAAGGTTTGAGGATGATCTGGACGAGGAGCGGGTTCCGA ATCTGGGGATAGTTGCCGAAAGTATTTTCGCAACTGGGTATCGACGTGCTGCT ATCGGGTGAGACCGGCACGGGCAAAGACACGATTGCCCGACGGATTTCATGAG ATGTCAGGCCGCAAAGGGCGCCTGGTGGCGATGAATTGCGCGGCCATTCCGG AGTCCCTCGCCGAGAGCGAGTTATTCGGCGTGGTCAGCGGTGCCTACACCGG CGCTGATCGCTCCAGAGTCGGTTATGTGAAGCGGCGCAGGGCGGCACGCTG TACCTGGATGAGATCGATAGCATGCCGCTGAGCCTGCAAGCCAAATTGCTGA GGGTGCTGGAAACCCGAGCGCTTGAACGGCTGGGTTCGACGTGACGATCAA GCTGGATATCTGCGTGATCGCCTCCGCCCCAATGCTCGCTGGACGACGCCGTC GAGCGGGGGCAGTTTCGTCGCGATCTGTATTTTCGCCTGAACGTCTTGACAC TCAAGCTTCCTCCGCTACGTAACAGTCTGATCGCATAGTTCCCCTGTTTAC ACGTTTTACGGCCGCCGCCGCGAGGGAGCTCGGTGTTCCCGTTCCCGATGTT TGCCCACTGCTGCACAAAGTGTGCTGGGCCACGACTGGCCCGGCAATATCC GTGAGCTCAAGGCGGCAGCCAAACGCCATGTGCTGGGTTTCCCTTGCTGGG CGCCGAGCCGAGGGCGAAGAGCACTTGGCCTGTGGGCTCAAATCGCAATTG CGAGTGATCGAAAAAGCCCTGATTCAGGAGTCGCTCAAGCGCCACGACAATT GTGTGGATTTCGGTAAGCCTGGAAC TGGACGTGCCACGCCGTACGCTCTATCG ACGCATCAAAGAATTGCAGATCTAATAA
<i>hrpV</i>	Gene ⁸⁻⁹	ATGATTGAGGTAACGAAAAAGTCGGCATTTCACGCTCAGGTTGCCGCCAGA GCCCAGCAGTATGGCCAGTGGCCAATGGCGTCGCGTTTGTTCAGTCGGCGCGA ACACCATGATTGGGGGATCGCCTTGACATAGAAGGCCGCGCGTTGCGTCCC GATCAACTGCGTGATGCACTGCAACGACGTTTTATGGAGTCAGAGCGTTTCA ATCACTACTTCCTGTTTCTGGACGTACGACGCGACTTTGTGGTGTGGCACGC GGTCAACGAAAAACCGGGTTCTACGCCAGCCTGGACGACATCCGCCGGCAT GAGTTGATGCTGGCAGGGCTGGACCATTTGAGCGAGGAAATGCACTAATAA
<i>hrpL</i>	Promoter ^{6,9}	GCCGGATTATGTCCGCTGAGTGGGTACGGTCCCGGATCAGTTCCCTTGCGA AGCTGACCGATGTTTTTGTGCCAAAAGCTGTTGTGGCAAAAAACGGTTTGCG CAAAGTTTTGTATTACAAAGAATTTACATTTTAAATATCTTTATAAATCA ATCAGTTATTTCTATTTTAAAGCTGGCATGGTTATCGCTATAGGGCTTGTAC

<i>P_{J101-rbs32-arsR-B0015-P_{arsR}}</i>	Inducible Promoter ¹⁰ (de novo synthesized)	TTTACAGCTAGCTCAGTCCTTGGTATTATGCTAGCTCCTAGGGTCACACAGG AAAGTACCATATGTCATTTCTGTTACCCATCCAATTGTTCAAAATTCCTTGCT GATGAAACCCGTCTGGGCATCGTTTTACTGCTCAGCGAACTGGGAGAGTTAT GCGTCTGCGATCTCTGCACCTGCTCTCGACCAGTCGCAGCCCAAGATCTCCCG CCACCTGGCATTGCTGCGTGAAAGCGGGCTATTGCTGGACCGCAAGCAAGGT AAGTGGGTTCATTACCGCTTATCACCGCATATTCCAGCATGGGCGGGCGAAAA TTATTGATGAGGCCCTGGCGATGTGAACAGGAAAAGGTTTCAGGCGATTGTCCG CAACCTGGCTCGACAAAACCTGTTCCGGGGACAGTAAGAACATTTGCAGTTAA AAATTTAGCTAAACACACATGAATTTTCAGATGTGTTTTATCCGGGTACTAG AGCCAGGCATCAAATAAAACGAAAGGCTCAGTCGAAAGACTGGGCCTTTTCGT TTTATCTGTTGTTTGTGCGTGAACGCTCTCTACTAGAGTCACACTGGCTCAC CTTCGGGTGGGCCTTTCTGCGTTTATATACCTAGAGCCAACTCAAAATTCACA CCTATTACCTTCCTCTGCACCTACACATTCGTTAAGTCATATATGTTTTTGA CTTATCCGCTTCGAAGAGAGACACTACCTGCAA
<i>araC-P_{BAD}</i>	Inducible Promoter ¹¹	TTATGACAACTTGACGGCTACATCATTCACTTTTTCTTACAACCGGCACGG AACTCGCTCGGGCTGGCCCCGGTGCATTTTTTAAATACCCGCGAGAAATAGA GTTGATCGTCAAAACCAACATTGCGACCGACGGTGGCGATAGGCGATCCGGGT GGTGCTCAAAAGCAGCTTCGCCTGGCTGATACGTTGGTCTCGCGCCAGCTT AAGACGCTAATCCCTAACTGCTGGCGGAAAAGATGTGACAGACGCGACGGCG ACAAGCAAACATGCTGTGCGACGCTGGCGATATCAAAATTGCTGTCTGCCAG GTGATCGCTGATGTACTGACAAGCCTCGCGTACCCGATTATCCATCGGTGGA TGGAGCGACTCGTTAATCGCTTCCATGCGCCGCAGTAACAATTGCTCAAGCA GATTTATCGCCAGCAGCTCCGAATAGCGCCCTTCCCCTTGCCCGGCGTTAAT GATTTGCCCAAACAGGTCGCTGAAATGCGGCTGGTGCCTTCATCCGGGCGA AAGAACCCCGTATTGGCAAATATTGACGGCCAGTTAAGCCATTATGCCAGT AGGCGCGCGGACGAAAGTAAACCCACTGGTGATACCATTGCGGAGCCTCCGG ATGACGACCGTAGTGATGAATCTCTCCTGGCGGGAACAGCAAAATATCACCC GGTCGGCAAACAAATTTCTCGTCCCTGATTTTTTCACCACCCCTGACCGCGAA TGGTGAGATTGAGAAATATAACCTTTTCATTCCCAGCGGTGCGTCGATAAAAAA ATCGAGATAACCGTTGGCCTCAATCGGCGTTAAACCCGCCACCAGATGGGCA TTAAACGAGTATCCCGGCAGCAGGGGATCATTTTGCGCTTCAGCCATACTTT TCATACTCCCGCCATTTCAGAGAAGAAACCAATTGTCCATATTGCATCAGACA TTGCCGTCACTGCGTCTTTTACTGGCTCTTCTCGCTAACCAGCCGTAACC CCGCTTATTAAAGCATTCTGTAAACAAAGCGGGACCAAGCCATGACAAAAA CGCGTAACAAAAGTGTCTATAATCACGGCAGAAAAGTCCACATTGATTATTT GCACGGCGTCACACTTTTGCTATGCCATAGCATTTTTATCCATAAGATTAGCG GATCCTACCTGACGCTTTTTATCGCAACTCTCT TACTGT TTCTCCAT
<i>rbs30</i>	RBS ³	TCTAGAGATTAAAGAGGAGAAATACTAGATG
<i>rbs32</i>	RBS ³	TCTAGAGTCACACAGGAAAGTACTAGATG
<i>J109</i>	Promoter ¹²	TTTACAGCTAGCTCAGTCCTAGGGACTGTGCTAGC
<i>J114</i>	Promoter ¹²	TTTATGGCTAGCTCAGTCCTAGGTACAATGCTAGC
<i>J116</i>	Promoter ¹²	TTGACAGCTAGCTCAGTCCTAGGGACTATGCTAGC
<i>J115</i>	Promoter ¹²	TTTATAGCTAGCTCAGCCCTTGGTACAATGCTAGC
<i>J105</i>	Promoter ¹²	TTTACGGCTAGCTCAGTCCTAGGTACTATGCTAGC
<i>J106</i>	Promoter ¹²	TTTACGGCTAGCTCAGTCCTAGGTATAGTGCTAGC
B0015 (B15)	Terminator ¹³	CCAGGCATCAAATAAAACGAAAGGCTCAGTCGAAAGACTGGGCCTTTTCGTTT TATCTGTTGTTTGTGCGTGAACGCTCTCTACTAGAGTCACACTGGCTCACCT TCGGGTGGGCCTTTCTGCGTTTATA

*gfp*Gene^{3,14}

ATGCGTAAAGGAGAAGAACTTTTCACTGGAGTTGTCCCAATTCTTGTGTAAT
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 TGATGCAACATACGGAAAACCTTACCCTTAAATTTATTTGCACTACTGGA
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 CCCTGTCTTTTACCAGACAACCATTACCTGTCCACACAATCTGCCCTTTTCG
 AAAGATCCCAACGAAAAGAGAGACCACATGGTCTTCTTGAGTTTGTACAG
 CTGCTGGGATTACACATGGCATGGATGAACATATACAAATAATAA

*pSB3K3*Plasmid
backbone¹⁵⁻¹⁶

TACTAGTAGCGGCCGTGCTGAGTCCGGCAAAAAACGGGCAAGGTGTCACCACCTGCC
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 TTCTGATTAGAAAACCTCATCGAGCATCAAATGAACTGCAATTTATTCATATCAGGA
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 CTTGTTCAACAGGCCAGCCATTACGCTCGTCAATCAAAATCACTCGCATCAACCAAACC
 GTTATTCAATTCGTGATTGCGCCTGAGCGAGACGAAATACGCGATCGCTGTAAAAGGA
 CAATTACAAACAGGAATCGAATGCAACCGGCGCAGGAACACTGCCAGCGCATCAACAA
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 TTAAGGCTAACTGAAAGGACAAGTTTTGGTGACTGCGCTCTCCAAGCCAGTTACCT
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 TATCAGAGGCAGAATTTAGATAAAAAAATCCTTAGCTTTTCGCTAAGGATGATTTT
 TGAATTCGCGGCCGCTTCTAGAG

Table S8: List of plasmid circuit constructs used in this study.

Plasmid	Description	Reference
pSB3K3	BioBrick vector, p15A ori, Kan ^r	iGEM Registry ¹⁵
pBW100ParsR	pSB3K3 carrying <i>J101-rbs32-arsR-t-P_{arsR}</i>	This study
pBW101ParsR-gfp	pSB3K3 carrying <i>J101-rbs32-arsR-t-P_{arsR}-30gfp-t</i>	This study
pBW102ParsR-Amp32 ^C	pBW100ParsR carrying <i>30hrpR-32hrpS-t-hrpL-30gfp-t</i>	This study
pBW103ParsR-Amp30 ^C	pSB3K3 carrying <i>J109-30hrpR-30hrpS-t-hrpL-30gfp-t</i>	This study
pBW110pBAD	pSB3K3 carrying <i>araC-P_{BAD}</i>	This study
pBW111pBAD-gfp	pSB3K3 carrying <i>araC-P_{BAD}-30gfp-t</i>	This study
pBW112pBAD-hrpV	pSB3K3 carrying <i>araC-P_{BAD}-30hrpV-t</i>	This study
pBW201J109-gfp	pSB3K3 carrying <i>J109-30gfp-t</i>	This study
pBW202J114-gfp	pSB3K3 carrying <i>J114-30gfp-t</i>	This study
pBW203J116-gfp	pSB3K3 carrying <i>J116-30gfp-t</i>	This study
pBW204J115-gfp	pSB3K3 carrying <i>J115-30gfp-t</i>	This study
pBW205J105-gfp	pSB3K3 carrying <i>J105-30gfp-t</i>	This study
pBW206J106-gfp	pSB3K3 carrying <i>J106-30gfp-t</i>	This study
pBW211J109-Amp32 ^C	pSB3K3 carrying <i>J109-30hrpR-32hrpS-t-hrpL-30gfp-t</i>	This study
pBW212J114-Amp32 ^C	pSB3K3 carrying <i>J114-30hrpR-32hrpS-t-hrpL-30gfp-t</i>	This study
pBW213J116-Amp32 ^C	pSB3K3 carrying <i>J116-30hrpR-32hrpS-t-hrpL-30gfp-t</i>	This study
pBW214J115-Amp32 ^C	pSB3K3 carrying <i>J115-30hrpR-32hrpS-t-hrpL-30gfp-t</i>	This study
pBW215J105-Amp32 ^C	pSB3K3 carrying <i>J105-30hrpR-32hrpS-t-hrpL-30gfp-t</i>	This study
pBW216J106-Amp32 ^C	pSB3K3 carrying <i>J106-30hrpR-32hrpS-t-hrpL-30gfp-t</i>	This study
pBW300ParsR-Amp32 ^T	pSB3K3 carrying <i>araC-P_{BAD}-30hrpV-t-J101-32arsR-t-P_{arsR}-30hrpR-32hrpS-t-hrpL-30gfp-t</i>	This study
pBW301ParsR-Amp30 ^T	pSB3K3 carrying <i>araC-P_{BAD}-30hrpV-t-J101-32arsR-t-P_{arsR}-30hrpR-32hrpS-t-hrpL-30gfp-t</i>	This study
pBW311J109-Amp32 ^T	pSB3K3 carrying <i>araC-P_{BAD}-30hrpV-t-J109-30hrpR-32hrpS-t-hrpL-30gfp-t</i>	This study
pBW312J114-Amp32 ^T	pSB3K3 carrying <i>araC-P_{BAD}-30hrpV-t-J114-30hrpR-32hrpS-t-hrpL-30gfp-t</i>	This study
pBW313J116-Amp32 ^T	pSB3K3 carrying <i>araC-P_{BAD}-30hrpV-t-J116-30hrpR-32hrpS-t-hrpL-30gfp-t</i>	This study
pBW314J115-Amp32 ^T	pSB3K3 carrying <i>araC-P_{BAD}-30hrpV-t-J115-30hrpR-32hrpS-t-hrpL-30gfp-t</i>	This study
pBW315J105-Amp32 ^T	pSB3K3 carrying <i>araC-P_{BAD}-30hrpV-t-J105-30hrpR-32hrpS-t-hrpL-30gfp-t</i>	This study
pBW316J106-Amp32 ^T	pSB3K3 carrying <i>araC-P_{BAD}-30hrpV-t-J106-30hrpR-32hrpS-t-hrpL-30gfp-t</i>	This study

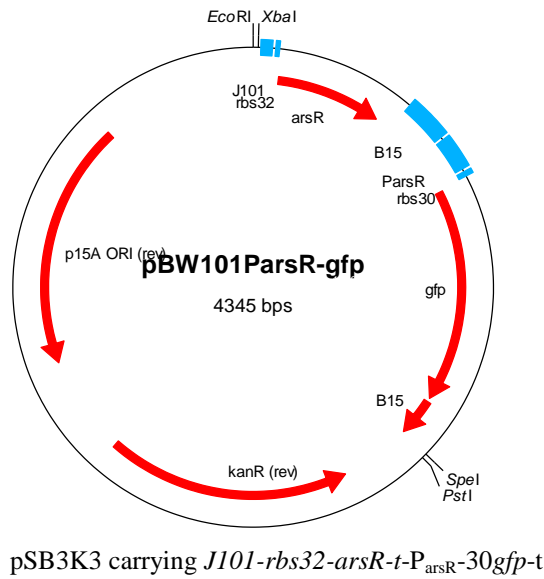
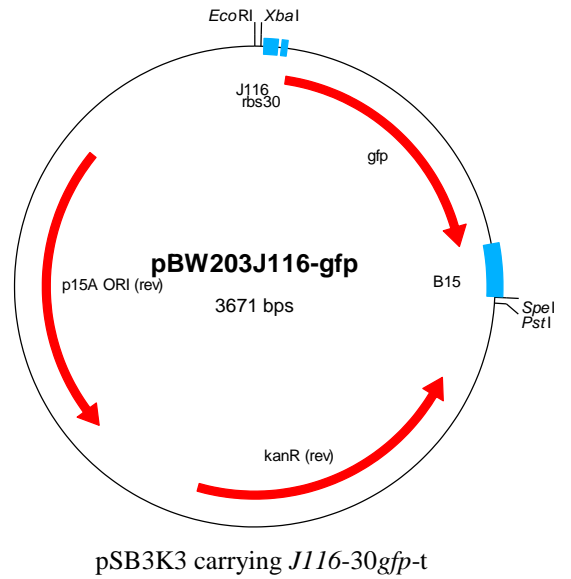
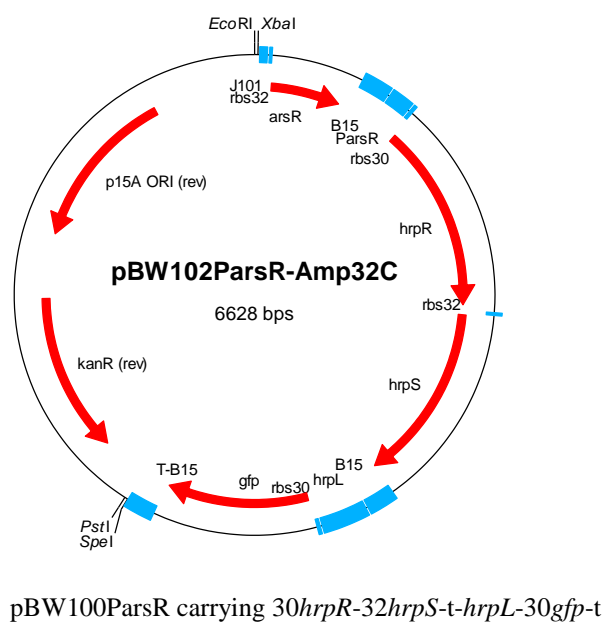
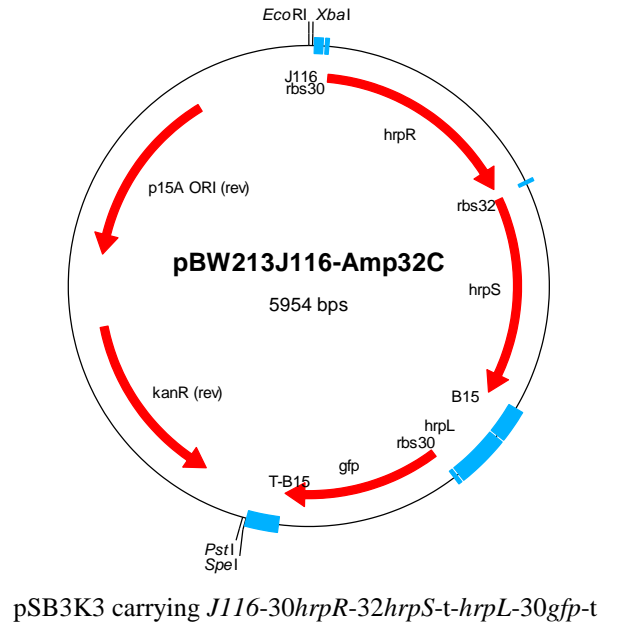
Promoter characterisation for input transfer functions**P_{arsR} input****P_C input (e.g J116)****Gain-fixed amplifier using P_{arsR} (left) or P_C (right) as the input****P_{arsR} input****P_C input (e.g J116)**

Figure S16: Plasmids used for the gain-fixed amplifier characterisation with the arsenic sensor input or constitutive promoter input. The top two plasmids were used for the characterisation of the arsenic responsive sensor input (Fig. S1, Fig. 2B) and constitutive promoter input (Fig. S5A). The bottom two plasmids were used for the fixed-gain amplifier Amp32^C characterisation (Fig. S2, Fig. 2A, Fig. 3D). To obtain Amp30^C, the rbs ahead of the *hrpS* gene was replaced by rbs30.

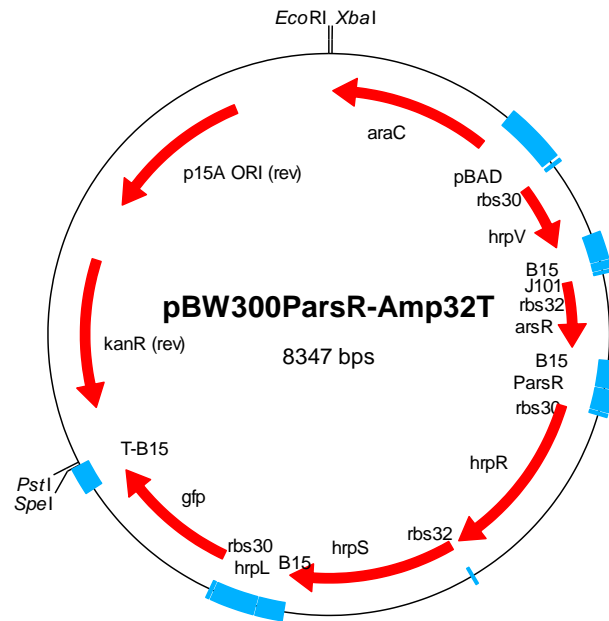
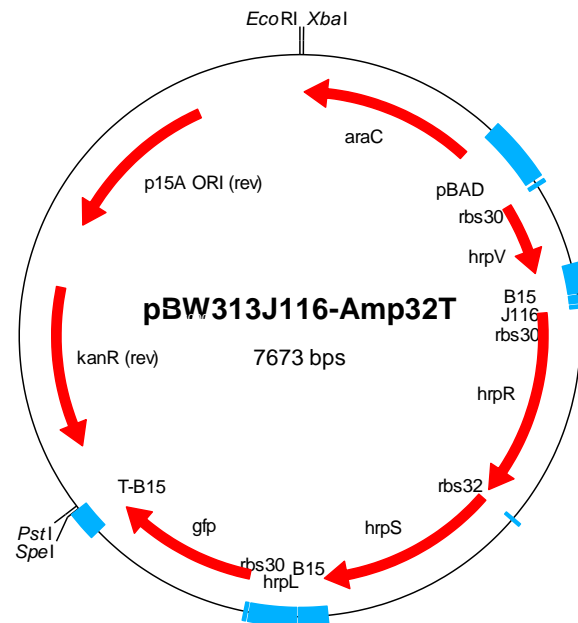
Gain-tunable amplifier using P_{arsR} or P_C as the input and P_{BAD} as the gain-tuning input**A****B**

Figure S17: Plasmids used for the gain-tunable amplifier with the arsenic sensor input or constitutive promoter input. (A) The plasmid for encoding the gain-tunable amplifier $Amp32^T$ with arsenic sensor as the input (Fig. 4A-D). (B) The plasmid for encoding the gain-tunable amplifier $Amp32^T$ with constitutive promoters (e.g. J116) as the input (Fig. S9). To obtain $Amp30^T$, the rbs ahead of the *hrpS* gene was replaced by *rbs30*.

Supplementary References

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